RESISTANCE OF CERTAIN STRAINS OF MICROORGANISMS TO ULTRAVIOLET RAYS

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The critical UV dose for 28 strains of microorganisms was evaluated. The aim was to select species suitable for experiments under Martian conditions. The most resistant to UV appeared to be nonpigmented spore forming bacteria and non-sporeforming yeast possessing pigments of intensive colorations (black, red). Microbial activity in the surface layers of the Martian soil seems to be quite probable judging from the data obtained.

In an artificial climate chamber developed and constructed in the Institute of Microbiology of the Academy of Sciences of the USSR, we conducted work on detecting forms, among terrestrial microorganisms, which are resistant to the set of physicochemical conditions characterizing the climate of the planet Mars. In the chamber, the following environmental parameters were created: pressure of 0.1 atm, a gas composition containing no oxygen, insolation approximating the complete solar spectrum in makeup, and temperature conditions changing from +25° to -60°C in the course of one day. /302*

It should be noted that -- in microbiological works of recent years carried out in chambers imitating various conditions -- it has been pointed out that the simultaneous effect of a number of factors differs from that of each factor taken separately. The papers of Silverman and Davis (1963) thus indicate that a vacuum of the order of 10^{-8} mm Hg protects the cells of Aspergillus niger, Bacillus stearothermophilus, Bac. megatherium, and Clostridium sporogenes from perishing under the effect of elevated temperatures. The same authors have demonstrated that when microorganism cells remain under an ultrahigh vacuum it increases their resistance to ionizing radiation (γ-rays of Co^{60}).

Working with representatives of higher planet life, Siegel et al. (1963) showed that the lack of oxygen in the gaseous mixture of the "Martian" air is favorable to germination of seeds at temperatures below zero.

From the sources in the literature it is known that none of the listed factors created in the artificial climate chamber kills microorganism cells even at extremes considerably exceeding those indicated above. It was found long ago that the temperature of liquid helium does not kill microbe cells (Becquerel, 1950), and a pressure of 10^{-2} mm Hg is used for the protracted preservation of bacterial cultures. The lack of oxygen is favorable to the development of anaerobic microflorae, representatives of which are widely disseminated under the most varied habitats on Earth. The effect of sunlight, however, especially that of the shortwave ultraviolet portion of the spectrum, on bacteria cells has received comparatively little study, although the bacteriocidic effect of ultraviolet rays is often used in the practice of medical institutions. It is also well known that even slight protection in the form

* Numbers in the margin indicate pagination in the original foreign text.

of a screen made of the most varied materials is enough to prevent the lethal effect of ultraviolet rays on microbe cells.

Preliminary experiments of short duration conducted in the artificial Martian climate chamber and previously reported (Zhukov and Kondrat'yev, 1965) established that irradiation is the chief factor limiting the viability of microorganism cells. Therefore, the most resistant species proved to be those with pigment of a protective coloration.

Since future experiments entail a study of the feasibility of terrestrial forms of microorganisms remaining for a protracted period and reproducing under artificial Mars conditions, the experiments on selecting the microorganism species most resistant to ultraviolet rays were continued. The work, however, was not conducted in the artificial climate chamber, whose "transmitting capacity" is very limited, but under a PRK-2 mercury lamp, in whose spectrum about 20% of all the energy is in the shortwave bacteriocidic portion. This is considerably more than the percentage of ultraviolet rays in the spectrum of the DKSSh-200 lamp. Taking into consideration the above information that a vacuum and an anoxygenic gaseous mixture increase total resistance of the cells, we must note that the forms selected in the course of the experiments will be more resistant to the action of the whole set of factors in the Martian chamber.

The basic experimental problems may be reduced to the following: /303

1. Detecting microorganism species most resistant to ultraviolet rays;

2. Determining the irradiation dosage which individual species of microorganisms can withstand; and

3. Forming an idea of the thickness of the screen protecting the cells from the lethal effect of ultraviolet rays.

Method

Since the moisture content of the Martian atmosphere is close to zero (Sharonov, 1958), the microorganism cells were placed under a PRK-2 lamp in a dry-air state on the surface of membrane bacteria filters. The cells were deposited on these filters by filtration of an aqueous suspension, which makes it possible to obtain a more uniform layer on the horizontal surface than do other methods -- for example, spraying. After irradiation, the filters were placed with their wrong sides on agar medium in Petri dishes, where they germinated at 28°C. The number of colonies on the dishes characterized the degree of the cells' viability after irradiation.

The cells of certain species of microorganisms and fungi do not give a uniform suspension, but form accumulations in the water in the form of small conglomerates. Subsequently, we used an ultrasonic device of the MSE brand (England) giving 16,000-24,000 cps per sec. to produce a suspension consisting of individual cells and free of conglomerations. Ultrasound was applied for

1 min., which, as testing showed, had no effect on the cells' viability, but prevented them from clumping.

The ultraviolet energy per square centimeter was determined by an ultraviolet-meter (UF-1, No. 9) measuring the photoflux in the wavelength region of 220-280 millimicrons. At the point where the experimental filters were placed, the ultraviolet energy was 14,000 erg/sec. This is about a third of the ultraviolet energy on the surface of Mars. In an experiment lasting 1 hr. the cells on the filter therefore received a dosage of $0.5 \cdot 10^8$ erg/sec of shortwave ultraviolet rays.

Results

Table I lists the species and indicates the dosage of ultraviolet energy at which these forms still maintain viability. At the bottom of the column are placed the microorganisms for which irradiation for 1 hr. was lethal. These experiments were conducted with preparations obtained after filtration of cell suspensions which had not been subjected to ultrasound. Twenty-eight species in all were analyzed in relation to ultraviolet. As has been previously remarked, the most resistant to the ultraviolet effect are pigmented species (Zhukova and Kondrat'yev, 1965). Therefore, primarily colored strains were analyzed. On the basis of experiments repeated many times, shown in Table I, the following conclusions may be drawn.

1. The tested forms of microorganisms, taken without regard to their physiological features, differ in their sensitivity to the effect of ultraviolet rays.

2. The most resistant proved to be colored /spores of fungi, pigmented yeast, and certain sporogenous forms of colorless bacteria.

3. Spore formation is apparently no less reliable a protection against ultraviolet action than is the presence of pigment -- since, in the last column in Table I listing the species which do not tolerate irradiation for 1 hr., all forms but one do not form spores. At the same time Bac. metaterium, unpigmented but sporogenous, is among the species most resistant to ultraviolet action.

The high ultraviolet dosages withstood by pigmented yeast and spores of fungi may, in all probability, be explained by the large number of cell conglomerations in the aqueous suspension. Even in cases where ultrasound acted for 2 min. on a suspension of these forms, it could not be completely freed of cell accumulations. From this we concluded that, to study the true reaction of cells of various species to ultraviolet ray action, we must deal with a single-layered preparation. Here the cells are arranged in one layer, do not shade each other, and are not protected from the direct action of the bactericidic rays. The production of guaranteed single-layer preparations requires that only those species be used which are completely freed of conglomerations after a short ultrasonic treatment which does not affect the viability of the experimental cells. Since suspensions of spores and black yeast did not possess this property in our experiments, they were not used to produce

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TABLE 1

EXTREME TOLERABLE LIMIT OF ULTRAVIOLET ENERGY DOSAGE FOR VIABILITY OF VARIOUS SPECIES OF MICROORGANISM

Tolerable UV Dose, erg/sq. cm	Microorganism Species	Spore Formation	Color of Colonies
$4.04 \cdot 10^8$	Torula nigra	No spores	Black
	Bac. megaterium	Spores	Colorless colonies
$3.02 \cdot 10^8$	Asp. niger	"	Black
	Asp. oryzae	"	Yellow-brown
	Mucor plumbeus	"	Black
	Bac. simplex	"	Colorless colonies
$2.02 \cdot 10^8$	Rhodotorula rubra	No spores	Red-pink
	Rhodotorula colostri	"	" "
$1.5 \cdot 10^8$	Tirotrix scaber	Spores	Colorless colonies
$1.01 \cdot 10^8$	Strain 1375*	No spores	Red-pink
	Bac. mycoides	Spores	Colorless colonies
	Bac. rubifaciens	"	" "
	Bac. lini	"	" "
$0.5 \cdot 10^8$	Strain 1551*	No spores	Red-pink
	Torulopsis glutinis	" "	Pink
	Micrococcus citreus	" "	Yellowish
	Bac. butyricus	Spores	Colorless colonies
	Bac. subtilis	"	Colorless colonies
	Micrococcus lysodeicticus	No spores	Yellow-green
	Micrococcus aurantiacus	" "	Pinkish
Species die off at irradiation of $0.5 \cdot 10^8$	Strain 1339*	" "	Red-pink
	Strain 1040*	" "	" "
	Strain 2230*	" "	Pink
	Rhodotorula glutinis	" "	Red-pink
	Photobacterium Krissi	" "	Colorless fluorescent colonies
	Serratia marcescens	" "	Colorless colonies
	Sarcina flava	" "	Yellow
	Flavobacterium aurantiacum	" "	Pale yellow

* Bacterial strains isolated from sea water.

monolayer preparations. "Single-layered" is the term we apply to a preparation in which the number of cells is such that their total area does not exceed the /305 area of the experimental field of the filter. Knowing the area of the visual field and also the area occupied by a single cell, we may prescribe in advance

a cell concentration -- in the aqueous suspension -- which, after filtration, leaves a single cell layer on the membrane filter. By doubling the cell concentration we have a two-layered preparation, and so on. All subsequent operations were conducted in accordance with the method described above.

The following experiments were aimed at determining the degree of protection against the bacteriocidic effect of ultraviolet rays offered by a screen made of the microbe cell bodies themselves. The reason such an experiment was set up was that, as a rule, microorganism cells are not found isolated in the atmosphere. The yeast Rhodotorula colostri proved to be the most suitable for these experiments. The total number of cells was determined by the method of direct counting on control filters. Irradiation lasted for 6 hr., i.e., the total dosage ($3 \cdot 10^8$ erg/sq. cm) deliberately exceeded that which this form was able to tolerate in the preliminary experiments.

Table II gives the results obtained in layering a homogeneous suspension of Rhodotorula colostri cells. From the table it is clear that incomplete "saturation" of the filter surface with cells, as a rule, leads to their death. An exception is the third version of the experiment. The growth of colonies on filters whose surface is not completely occupied by the tested cells may be attributed to several cells getting on top of the filtered cells in the first moment of filtration (usually of 2 to 3 cc of suspension). Therefore the results of estimating the cultured colonies also differs greatly in the various versions of the experiment. Doubling the single-layered quantity of cells increases the number of viable cells by a factor of four or more.

To speak of the absolute degree of protection against ultraviolet rays by a single layer is for the time being difficult due to the above-mentioned methodological difficulties, but the data presented indicate that a screen of a single layer of Rhodotorula colostri reduces the bacteriocidic properties of the ultraviolet rays from a PRK-2 lamp by a factor of at least four. This gives an idea of the slight penetrability by ultraviolet rays of a layer of organic material of bacterial cells 1.9 microns thick (the diameter of a single cell of Rhodotorula colostri). Moveover, this makes it possible to /306
imagine that the experimental conglomerations of cells -- so disposed that the inside cells have protection at least equal to the thickness of a single adjacent cell -- will allow the colony to grow, since the bacteriocidal effect of ultraviolet rays will be reduced by a factor of four and will not exceed the dosage tolerable by cells of this species (Table 1). Consequently, in experiments aimed at deriving clear findings as to the viability of cells in an artificial climate chamber, single-layered preparations of live cells must be used.

Conclusions

1. For the purpose of selecting species suitable for protracted experiments in an artificial Martian climate chamber, we determined the extreme tolerable dosage of ultraviolet for twenty-eight strains of microorganisms.

2. The most resistant to ultraviolet ray action proved to be colorless sporogenous forms of bacteria and non-sporogenous yeast having pigment of strong coloration (black, red).

TABLE II

PROTECTIVE EFFECT OF CELLS OF RHODOTORULA COLOSTRI AGAINST ULTRAVIOLET RAYS (IRRADIATION DOSAGE $3 \cdot 10^8$ erg/sq. cm)

Version of Experiment	Number of Cells in Visual Field	Area of Visual Field Occupied by Cells, %	Number of Colonies Cultured on Filters
I	1362	50.6	0
	2724	105	4
	5448	201	107
II	1596	59	0
	3364	124	3
	4960	184	27
	6728	250	109
III	1356	50	25
	2712	100	36
	4068	155	39
IV	724	27	0
	1680	62	0
	2828	102	22

3. To study the degree of penetrability by ultraviolet rays through the bodies of bacterial cells we developed a method of producing single-layered bacterial preparations.

4. It has been established that the screen consisting of a single layer of Rhodotorula colostri cells reduces the bacteriocidic effect of ultraviolet rays by a factor of at least four.

5. On the basis of these experiments it must be considered that it is entirely probable that there is microbe activity in the surface layers of the "soil" of Mars.

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